

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries
AUTHORS	Peter Aaby, Christine Stabell Benn, Jens Nielsen, Ida Maria Lisse, Amabelia Rodrigues and Henrik Ravn

VERSION 1 - REVIEW

REVIEWER	Frank Shann Professor of Critical Care Medicine Royal Children's Hospital, Melbourne, Australia University of Melbourne, Australia.
REVIEW RETURNED	30/12/2012

THE STUDY	I suggest the authors cite their paper: Agergaard, vaccine 2011;29:487-500.
GENERAL COMMENTS	<p>This is a useful discussion of a complicated topic that brings together many different strands of evidence in an elegant manner. However, the paper could be improved.</p> <p>DTP after nothing, after BCG, and after MV -----</p> <p>I think the authors need to emphasise that DTP may well have very different non-specific effects depending on whether it is given to previously unvaccinated children (harmful to girls, less or no harm to boys - reference 42); given at least 4 weeks after measles vaccine (MV) (perhaps harmful for girls, perhaps beneficial for boys - see under F/M ratios, below); given at least 4 weeks after BCG (harmful for girls and unclear for boys - references 64 and 69, and Shann, J Infect Dis 2011;204:182-4); given at the same time as BCG or MV (effects unclear); and given after vitamin A.</p> <p>Emphasise the strongest evidence -----</p> <p>I think the strongest evidence that DTP increases mortality relates to its administration after BCG (rather than after measles vaccine). This comes from reference 64, and from an analysis of DTP-then-BCG versus BCG-then-DTP versus Controls (DTP only) in reference 69 (see Shann, J Infect Dis 2011;204:182-4). And yet these two references are mentioned only in passing in this paper. In addition, reference 42 is the only study of the effect of DTP on mortality when it was first used in a community with no herd immunity - and risk-adjusted all-cause mortality was increased by 1.92 (1.04-3.52), with a crude mortality of 11.3% after DTP and 5.1% in controls.</p> <p>Female/male mortality ratios</p>

I think the authors put too much emphasis on Female/Male mortality ratios (F/M ratios). It is not crucially important to know whether DTP has a different effect in girls and boys, but it is crucially important to know whether DTP causes harm to girls or boys.

An increase in the F/M ratio could be due to a reduction in male and female mortality (male more than female), an increase in male and female mortality (female more than male), a reduction in male mortality, or an increase in female mortality, or a combination of these - without being told the underlying mortality rates we do not know the cause, so just stick with rates. At the very least, the underlying rates should be given for every ratio and for Supplementary Tables 2, 3 and 4.

For EVERY increased F/M ratio, the onus is on the authors to show that the increase is due to an increase in female mortality rather than a reduction in male mortality (for example, for Figures 5-7, and Supplementary Tables 1,3-5). There is strong evidence that DTP increases mortality after BCG (Shann, J Infect Dis 2011;204:182-4), but only weak evidence that DTP is harmful after MV. Indeed, there is evidence that, until the next vaccine is given, DTP may LOWER mortality in boys when it is given after MV:

- in reference 5, Table 3, boys had a mortality rate of 2.9% when MV at 9-10 months was followed by DTP-IPV or IPV, and 5.0% when MV was not followed by DTP-IPV or IPV (and girls had a mortality rate of 4.5-5.2% whether or not MV was followed by DTP). This may be due to selection bias, but the resulting high F/M ratio should not be used by the authors as evidence for an adverse effect of DTP in girls after MV.
- in reference 5, Table 4, boys had a mortality rate of 1.9% when MV at 4 months was followed by DTP, and 4.7% when MV was not followed by DTP (and girls had a mortality rate of 4.2-4.7% whether or not MV was followed by DTP). This may be due to selection bias, but the resulting high F/M ratio should not be used by the authors as evidence for an adverse effect of DTP in girls after MV.
- in reference 22, page 2767, when MV was followed by DTP in boys the mortality ratio was 0.69 (0.20-2.33) compared to when MV was not followed by DTP.
- in reference 31, Table 2, the mortality rate for boys aged 5-8 months (during and after DTP) was 40 per 1000py, compared to 67-70 per 1000py for girls of the same age as well as younger and older boys
- 6 months after randomisation, boys randomised to receive DTP (with MV and OPV) were heavier and had better weight-for-height than boys randomised to receive only MV and OPV (Agergaard, Vaccine 2011;29:487-500). Why is this randomised trial of DTP not mentioned in the paper?

All the relevant studies (including some of the above) should be included in Supplementary Table 6. Do the authors know of any other evidence that DTP-after-MV may lower mortality in boys?

The authors advance four reasons why reduced male mortality after DTP does not fully explain their findings (Discussion, page 12). Rather than deal with each reason in turn, I suggest that there would be no reason to use F/M ratios at all if the authors demonstrated convincingly their four propositions: (1) DTP increases overall mortality, (2) female mortality was increased after HTMV (though this alone does not incriminate DTP), (3) DTP after MV increases mortality

in girls (I don't think it does), and (4) MV after DTP reduces mortality in girls PROVIDING THEY HAVE NOT RECEIVED VITAMIN A (but this does not prove that DTP increases mortality, it merely confirms that MV reduces mortality - in both girls and boys, reference 3). The authors' fifth point is merely a rephrasing of their fourth point. I would add, (5) DTP increases mortality in girls and boys after BCG (which is crucially important because this is recommended in the EPI schedule). It is important to emphasise that the non-specific effects occur until the next vaccine is given. It would be better to demonstrate these points clearly and directly (especially points 4 and 5, because they have very important implications for the EPI schedule), without resorting to F/M ratios - which are not convincing.

The authors often confuse an increased F/M ratio with increased female mortality. For example, in the Introduction page 4 line 12, reference 5 reports a F/M ratio of about two (which was because of a lower mortality rate in boys who received DTP after HTMV, Table 4) and not a "two-fold increased female mortality" (in reference 5, Tables 2 and 4, female mortality was not higher in the HTMV group between 4 and 10 months). Figure 3 (DTP Observation 4) also states that mortality after HTMV was increased only in girls who had received DTP or IPV after HTMV, and cites reference 5.

Non-specific effects of BCG and MV

I suggest that it would be worth devoting a paragraph to the very strong evidence that, until the next vaccine is given, BCG (references 78, 65 and Shann, Arch Dis Child 2010;95:662-7) and MV (references 3 and Shann, Arch Dis Child 2010;95:662-7) reduce mortality from diseases other than tuberculosis and measles. Of course, this paper is about DTP and not BCG or MV, but the strong evidence that BCG and MV have non-specific effects sets the scene for DTP having similar actions.

Other matters

In the Abstract (page 2 line 7), I suggest "We examined whether whole-cell diphtheria-tetanus-pertussis vaccine (DTP) has ..." In the Data Sources section (page 2, line 10), I suggest "The effect of DTP on mortality up to the next vaccination was assessed in all studies where DTP was given after BCG and there was prospective follow-up after ascertainment of vaccination status." Under Setting (page 2, line 13, I think "high-mortality" is preferable to "low-income"; vaccines have important non-specific effects on mortality only in high-mortality regions (where most deaths are caused by infection), and some countries with a low income have low child mortality (for example, Vietnam and Sri Lanka, as well as Kerala in India).

On page 4 line 32, reference 78 does not make any comment about the ethics of randomised trials of DTP. However, the report of the WHO Task Force on Routine Infant Vaccination and Child Survival that met in London in May 2004 states that "Indeed, the task force considered that some trial designs, such as placebo-controlled or studies involving delay in DPT vaccination, would be unethical." - see www.who.int/entity/vaccine_safety/topics/dtp/taskforce_report.pdf.

On page 5 line 54, I agree with the authors that comparisons of sequential vaccinations should be interpreted cautiously, but I do not

agree that this is sufficient reason to emphasise the comparison of female and male mortality rates (in EVERY case, the authors have to demonstrate that a high F/M ratio is not due to reduced mortality in boys).

Supplementary Table 2 is confusing because (1) it presents only mortality ratios, and (2) it is not clear what groups are being compared - for example, are the DTP ratios DTP1 to unvaccinated, DTP1 to BCG (or MV), DTP1 or DTP2 or DTP3 to unvaccinated or BCG (or MV)? The underlying rates should be reported where possible, and the groups being compared should be described.

On page 11 line 56, in the discussion about BCG revaccination, we do not know whether there was a reduction in admissions for girls or an increase for boys - because the text (and reference 69) give only ratios (we should be told RATES).

In the Discussion (page 12, line 25) it is claimed that the two natural experiments suggested an increased mortality in boys, but I am not sure that this statement is justified by the papers cited. In reference 42, the mortality for DTP versus no DTP in boys was 1.56 (0.70-3.48), which is consistent with a 30% REDUCTION in mortality. The second study (reference 43) presents no data by sex - what was the estimate for boys, and does the 95% CI include 1.00?

On page 14, line 53, I suggest that delaying DTP until just before MV at 9 months may not be as effective as giving DTP as at present, but giving MV earlier (just after the three primary doses of DTP). This would continue to give early protection against diphtheria, tetanus and pertussis, and may increase the beneficial non-specific effects of MV (if they are enhanced by the presence of maternal antibodies); a booster dose of MV could then be given after the DTP booster in the second year of life to reverse the adverse effect of DTP and increase protection against measles. It would therefore be of very great interest to randomise children to receive either (1) the standard EPI schedule of BCG at birth, DTP at 6, 10 and 14 weeks, MV at 9 months, and DTP booster at 18 months, or (2) BCG at birth, DTP at 6, 10 and 14 weeks, MV at 18 weeks, DTP booster at 18 months, and MV at 19 months. Now that a booster dose of DTP is recommended in the second year of life, two primary doses of DTP might be enough, which would allow the first dose of MV to be given even earlier (when more infants would have maternal antibody). This suggests randomising to (1) the standard EPI schedule, versus (2) BCG at birth, DTP at 6 and 10 weeks, MV at 14 weeks, DTP at 18 months, and MV at 19 months.

References 27, 51 and 53 have the wrong titles. I have not checked all the references, but this needs to be done.

Conclusion

This is an important study of an extremely important topic. It is outrageous that so little attention has been paid to the evidence that DTP is causing serious harm in high-mortality regions. However, the paper would be greatly improved by abandoning the use of F/M ratios; at the very least, the underlying mortality rates should be given whenever F/M ratios (or mortality ratios) are used. In addition, I suggest it would be a good idea to mention the strong evidence that BCG and MV have very large beneficial non-specific effects, and to give more emphasis to the strongest evidence that DTP has harmful

	<p>non-specific effects in girls in high-mortality regions.</p> <p>There is strong evidence that (1) BCG has beneficial non-specific effects, (2) DTP has harmful non-specific effects when it is given after BCG (as specified in the EPI schedule), and (3) MV has beneficial non-specific effects (for both boys and girls) when it is given after DTP. However, there is only weak evidence that DTP is harmful (for girls) when it is given after MV - and yet that is the interaction the authors have emphasised (it is a weak or non-existent interaction, and the EPI schedule does not recommend giving DTP to infants after MV anyway - though a booster dose of DTP in the second year of life would be given after MV). I would revise the paper to emphasise these points, but this is merely a tentative suggestion that the authors may wish to ignore.</p>
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REVIEWER	Tobias R. Kollmann University of British Columbia
REVIEW RETURNED	02/02/2012

The reviewer completed the checklist but made no further comments.

REVIEWER	<p>Robert T Chen MD Medical Epidemiologist Clinical Trials Team Division of HIV/AIDS Prevention CDC, Atlanta</p> <p>I have no competing interests</p>
REVIEW RETURNED	14/02/2012

THE STUDY	This is essentially a review of previously published papers, so other than main study design, little details of the original papers are presented.
RESULTS & CONCLUSIONS	This paper presents the results of two of six recommended trials recommended by the Working Group on Non-Targetted Effects. So while the results to date are supportive, one can not say the research question has been "answered" until the other trials are done.
GENERAL COMMENTS	<p>* Such faith/religious belief in "absolute good" of immunizations is problematic as:</p> <ol style="list-style-type: none"> 1) Vaccines are simply a pharmaceutical product where ideally, recommendations for its use should be based on a dynamic assessment of its benefits, costs, and risks. 2) Given likely Gaussian distribution of genetic diversity in the population, some vaccinees will underrespond and some over-respond, with vast majority in the middle. 3) Almost all manufactured goods can only improve by a virtuous cycle of problem/error detection feeding back to problem solving rather than assuming it is already perfect from initial introduction. <p>General comments:</p> <p>This is an unusual paper in several ways.</p> <ol style="list-style-type: none"> 1) The authors (Aaby et al) have summarized in detail a decade-long personal and very uphill quest to explain an unexpected scientific finding: that child survival in certain pediatric vaccinees in low income countries (LIC) is unexpectedly lower than the comparison groups. Due to skepticism over just compilation of observational study findings alone, consistent with recommendations of the

	<p>Working Group on Non-Targeted Effects, they have recently used small randomized trials to test their hypotheses (with generally supportive findings).</p> <p>2) The larger vaccine community has been generally reluctant to take the Aaby hypothesis seriously, leading many to simply dismiss the findings without examining the presented evidence or offering alternative hypotheses. The reasons for this dismissal may include:</p> <ul style="list-style-type: none"> a. The standard belief/simplification that if vaccines and immunizations are good for public health, they must be an absolute (vs. relative) good. Those who cast any doubts on this absolute good (e.g., Aaby et al) must be either ignorant or “anti-vaccine”. * b. The findings are difficult to believe/understand: <ul style="list-style-type: none"> i. Study settings among the poorest in the world (e.g., Guinea Bissau, Haiti, Bangladesh): <ul style="list-style-type: none"> 1. Difficult to organize good studies logistically 2. Many “bad” things occur in these settings leading to poor child survival anyways. 3. Mortality is a crude final outcome with many possible causes, especially in LDC settings. 4. Difficult to find true peer reviewers. Those that exist know painfully how easy studies can go wrong in such settings and therefore “truth” more elusive. ii. These “non-targeted” effects differ by gender (worse in females) for unknown reasons. iii. Lack of a known biologic mechanism to explain the findings. iv. Dr. Aaby is a demographer by training (vs. medicine or public health), and therefore outside of the usual “fraternity”. c. Few others have been willing (to fund) or replicate the study findings. Therefore the authors are by and large the only ones working on this question, further aggravating the dismissal/credibility problem. This is despite many new ongoing and planned clinical trials * Such faith/religious belief in “absolute good” of immunizations is problematic as: <ul style="list-style-type: none"> 1) Vaccines are simply a pharmaceutical product where ideally, recommendations for its use should be based on a dynamic assessment of its benefits, costs, and risks. 2) Given likely Gaussian distribution of genetic diversity in the population, some vaccinees will underrespond and some over-respond, with vast majority in the middle. 3) Almost all manufactured goods can only improve by a virtuous cycle of problem/error detection feeding back to problem solving rather than assuming it is already perfect from initial introduction. <p>of candidate vaccines vs. poverty-related diseases (PRD) like dengue, malaria, TB in LICs where both routine pediatric vaccination and child survival are tracked.</p> <p>Specific Comments:</p> <p>3) Introduction: Suggest rewording “observational studies have little impact” to “observational studies have historically rank lower in grade of evidence for quality of study design than randomized studies (ref: PMID 10861325). In the Discussion, the authors may want to point out that this assumption has been questioned by some (PMID:19463302), as this ref (and PMID 10861324) suggest, the results of well-designed observational studies are usually highly concordant with that of the RCTs.</p> <p>4) For all findings with a significant positive or negative association between the vaccine and the outcome of interest, it would be helpful if the analysis examined if there is a non-random clustering of the time interval (e.g., in weeks) between vaccination and the adverse event or not (e.g., febrile seizures cluster ~1 week after live viral vaccines).</p>
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	<p>5) For the DTP studies, was acellular pertussis vaccine used at all; if so, does the non-targeted effects differ between wP and aP vaccine? The answer may provide some insights into the mechanism of action.</p> <p>6) If some of the databases used in the analyses are available for public use, it may allow other researchers (e.g., doctoral students) to confirm the analyses and findings.</p> <p>7) The Working Group on Non-Targeted Effects (WGNT, Ref 104) had recommended at least six possible trials to assess the nonspecific effects of vaccines on mortality. This paper includes the outcomes of two of the six trials. Please mention Ref 104 earlier in the paper (e.g., in Section B. Testing the Hypothesis) so the reader is aware of these six trials and how the two fit within that context. In the Discussion, please also discuss the status of the remaining recommended trials and why we shouldn't await their results before acting.</p> <p>8) Rather than the authors submitting the current paper, the impact on policymakers may be greater if the recommendations came from the independent WGNT, especially after the results of more than two of their recommended trials are available.</p> <p>9) As noted above, there are many planned trials of candidate vaccines vs. PRD in LIC that will also track child survival as an outcome. The authors may wish to suggest how these researchers can integrate testing of non-target effects hypotheses into such trials.</p> <p>10) Table 1B, suggest rewording to: "Studies not used in the analysis due to simultaneous vaccination (see text)"</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer: Frank Shann
Professor of Critical Care Medicine
Royal Children's Hospital, Melbourne, Australia University of Melbourne, Australia

I suggest the authors cite their paper: Agergaard, vaccine 2011;29:487-500.

PA: This paper was cited as reference 77 but it was not in focus since we excluded the papers in which DTP and measles vaccine (MV) were administered simultaneously.

This is a useful discussion of a complicated topic that brings together many different strands of evidence in an elegant manner. However, the paper could be improved.

DTP after nothing, after BCG, and after MV

I think the authors need to emphasise that DTP may well have very different non-specific effects depending on whether it is given to previously unvaccinated children (harmful to girls, less or no harm to boys - reference 42); given at least 4 weeks after measles vaccine (MV) (perhaps harmful for girls, perhaps beneficial for boys - see under F/M ratios, below); given at least 4 weeks after BCG (harmful for girls and unclear for boys - references 64 and 69, and Shann, J Infect Dis 2011;204:182-4); given at the same time as BCG or MV (effects unclear); and given after vitamin A.

PA: We have now emphasised that the sequence in which DTP is given and whether vitamin A had also been given may influence the overall effect. This information has been provided in all tables. Furthermore we have added a table in the discussion (Table 2) summarising the evidence for overall and sex-differential effects of DTP when given as first vaccination, when given after BCG, and when

given after MV.

Emphasise the strongest evidence

I think the strongest evidence that DTP increases mortality relates to its administration after BCG (rather than after measles vaccine). This comes from reference 64, and from an analysis of DTP-then-BCG versus BCG-then-DTP versus Controls (DTP only) in reference 69 (see Shann, J Infect Dis 2011;204:182-4). And yet these two references are mentioned only in passing in this paper. In addition, reference 42 is the only study of the effect of DTP on mortality when it was first used in a community with no herd immunity - and risk-adjusted all-cause mortality was increased by 1.92 (1.04-3.52), with a crude mortality of 11.3% after DTP and 5.1% in controls.

PA: Reference 64 was not published when this paper was first submitted and therefore not emphasised as strongly as suggested by professor Shann. Ref 69 was mentioned in relation to the RCT reducing exposure to DTP so it had a long paragraph in section B of the paper. All three papers have now been more strongly emphasised and a paragraph on “the strongest evidence” has been added to the discussion which highlighten references 42, 64, and 69. We would also emphasised reference 43 and the studies of DTP after both high-titre and standard titre MV (Supplementary Table 6).

Female/male mortality ratios

I think the authors put too much emphasis on Female/Male mortality ratios (F/M ratios). It is not crucially important to know whether DTP has a different effect in girls and boys, but it is crucially important to know whether DTP causes harm to girls or boys.

PA: We may not have been sufficiently clear in how the subject was presented. We are using two approaches: A) comparing the relative mortality estimates for different vaccines, and B) the relative estimates for girls and boys who have received the same vaccination. The A comparisons serve to document that there is something wrong with DTP because – while we should expect that DTP coming after BCG should be associated with lower mortality than BCG - it is in fact associated with higher mortality. The B comparisons serve to document that this adverse effect of DTP is systematically worse for girls than for boys – since the trends are the opposite for BCG and MV with beneficial efficial effects for girls, the negativ effect of DTP for girls can not simply be explained as the way girls are treated. In relation to the hypothesis tested both types of comparisons are needed.

An increase in the F/M ratio could be due to a reduction in male and female mortality (male more than female), an increase in male and female mortality (female more than male), a reduction in male mortality, or an increase in female mortality, or a combination of these - without being told the underlying mortality rates we do not know the cause, so just stick with rates. At the very least, the underlying rates should be given for every ratio and for Supplementary Tables 2, 3 and 4.

PA: The rates or case fatalities have now been given in the tables where only ratios had been provided, i.e supplementary tables 2-5. However, the F/M MRR have been maintained because they report a comparison of children who have been treated in the same way (i.e. received the same vaccines) and therefore presumably shares the same selection biases (see above). Comparing the DTP-vaccinated and DTP-unvaccinated children is comparing groups which have been treated in very different ways. The complications entailed in this kind of comparison are evidenced from our comments below. Our paper now more strongly empasises that both the analyses of the differential effects of different vaccines and the studies of the sex-differential effects of the same vaccine for boys

and girls suggest that DTP is associated with increased mortality – i.e increased mortality for girls.

For EVERY increased F/M ratio, the onus is on the authors to show that the increase is due to an increase in female mortality rather than a reduction in male mortality (for example, for Figures 5-7, and Supplementary Tables 1,3-5).

PA: For each observation on sex-differential effects we have added whether there is reliable information to indicate whether the effect is due to an increase in female mortality or a reduction in male mortality.

There is strong evidence that DTP increases mortality after BCG (Shann, J Infect Dis 2011;204:182-4), but only weak evidence that DTP is harmful after MV. Indeed, there is evidence that, until the next vaccine is given, DTP may LOWER mortality in boys when it is given after MV:

PA: There is strong evidence (Observation V and VI, Supplementary Table 6) that DTP after MV enhances female mortality. There is no convincing evidence that DTP after MV may lower mortality in boys (see comments below).

- in reference 5, Table 3, boys had a mortality rate of 2.9% when MV at 9-10 months was followed by DTP-IPV or IPV, and 5.0% when MV was not followed by DTP-IPV or IPV (and girls had a mortality rate of 4.5-5.2% whether or not MV was followed by DTP). This may be due to selection bias, but the resulting high F/M ratio should not be used by the authors as evidence for an adverse effect of DTP in girls after MV.

PA: All data for an apparent benefit of getting DTP after MV for boys comes from the trial in Senegal. In Senegal the children not getting DTP after MV were those who were travelling or abstained and therefore did not get the benefit of the children attending the vaccination session at 10 months of age. These benefits included antibiotic treatment plus drugs for malaria prophylaxis for several months. Hence, it is not surprising that children attending the vaccination sessions to get DTP after MV had lower mortality as observed among the boys. The really surprising part of this study was that the girls who attended and got DTP after MV had slightly higher mortality than the girls who did not attend (ref 5).

- in reference 5, Table 4, boys had a mortality rate of 1.9% when MV at 4 months was followed by DTP, and 4.7% when MV was not followed by DTP (and girls had a mortality rate of 4.2-4.7% whether or not MV was followed by DTP). This may be due to selection bias, but the resulting high F/M ratio should not be used by the authors as evidence for an adverse effect of DTP in girls after MV.

PA: Ref 5, Table 5 can not be used in this way. The table was constructed to measure the effect on the relative mortality of girls and boys among children receiving DTP after MV or not receiving DTP after MV for groups who had received the same treatment. The season at risk and the age of vaccination may be different between the children who received or not received DTP after MV. Most important the children who died before they received DTP after MV would come in the group which received no DTP after MV whereas those surviving to get additional doses would come in the group of additional DTP after MV. On this background it is not surprising that mortality rates are lower among those getting DTP after MV.

In our view the best data for the effect of DTP after MV are those summarised in Supplementary Table 6 and Figure 8. Girls likely to get DTP after MV had more than two fold high mortality than those not likely to get DTP after MV whereas this difference had no effect for boys. These data shows clearly a sex-differential effect of DTP after MV and suggest also increased mortality for DTP after MV for girls – and no difference for boys.

- in reference 22, page 2767, when MV was followed by DTP in boys the mortality ratio was 0.69 (0.20-2.33) compared to when MV was not followed by DTP.

PA: There is a misunderstanding here. Table 2, page 2767 in this paper is about the relative proportion of children having received DTP3 before enrolment in various subgroups in the Sudanese trial. At the same time the table report the relative mortality in the subgroups examined. Hence, the ratio of 0.69 (0.20-2.33) is the mortality ratio of boys versus girls within the whole trial and has nothing to do with getting DTP after MV. The data from the Sudan study for getting DTP after MV are reported in Supplementary table 6 and the MRR for likely to get DTP/not likely to get DTP after MV was 2.16 for girls and 0.71 for boys.

- in reference 31, Table 2, the mortality rate for boys aged 5-8 months (during and after DTP) was 40 per 1000py, compared to 67-70 per 1000py for girls of the same age as well as younger and older boys

PA: Yes these data which are also presented in Figure 10 suggests that the mortality of boys may increase around 9 months of age when they receive MV after DTP. However, these data do not say anything about receiving DTP after MV for either boys or girls. Please note in the curve in figure 10 that after 18 months of age where DTP has been given in Gambia mortality curves appears to be stable (boys) or increase (girls) in spite of the fact that one would expect the mortality rate to decline with age. Again the best data on this problem is the data in supplementary table 6 where there is no indication that DTP after MV reduces mortality for boys.

- 6 months after randomisation, boys randomised to receive DTP (with MV and OPV) were heavier and had better weight-for-height than boys randomised to receive only MV and OPV (Agergaard, Vaccine 2011;29:487-500). Why is this randomised trial of DTP not mentioned in the paper?

PA: This study (ref 77) can not be used to say anything about the effect of DTP after MV in boys. As mentioned above we excluded this study along with other studies dealing with simultaneous administration of DTP and MV. The study is mentioned but as an example of a study dealing with simultaneous administration of DTP and MV.

All the relevant studies (including some of the above) should be included in Supplementary Table 6.

PA: Supplementary Table 6 includes MV trials where the DTP status was assessed at the time of enrolment which permits us to assess whether receiving or not receiving DTP after MV affects the mortality level for both boys and girls. The papers from Senegal and Agergaard to which professor Shann refers above are not of this type as they administered MV and DTP simultaneously. Furthermore, the high-titre trial studies in reference 5 from Senegal provided all doses of DTP within the trial set up. Hence, whether a child received DTP after MV or did not receive DTP is related to whether the mother was compliant and brought the child back – and this difference entailed a lot of selection biases as discussed above. With one exception all the published relevant papers are included in the table. The one exception is the two small trials from Bissau in 1980s in which virtually none of the children had received DTP3 before enrolment and these trials were therefore omitted in the first presentation. However, we have reported the results in reference 51 and it may therefore be more logical to include them also in the present table. Furthermore, we have recently submitted an analysis of the last MV trial from Bissau which also has relevant information on the effect of DTP after MV. Table 6 has been modified accordingly. The modification makes the difference in effect for boys and girls significantly different. The table now has data from all four MV trial from Bissau in which it was not required that the children had received DTP3 before enrolment.

In the presentation of Observation V we have tried to explain the context more carefully.

Do the authors know of any other evidence that DTP-after-MV may lower mortality in boys?

PA: We do not know of further studies than those presented in Supplementary Table 6 and Observation IV-V which may indicate whether DTP after MV is associated with lower mortality for boys. Please note the comment about the effect of booster DTP after 18 months of age in the Gambia. In the graph below similar data can be seen from Guinea-Bissau in the 1990s when booster DTP was provided around 18 months of age. Instead of declining as expected the mortality rate increased for both boys and girls after 18 months of age. These data do not support the proposition that boys might have lower mortality when DTP is given after MV.

The authors advance four reasons why reduced male mortality after DTP does not fully explain their findings (Discussion, page 12). Rather than deal with each reason in turn, I suggest that there would be no reason to use F/M ratios at all if the authors demonstrated convincingly their four propositions: (1) DTP increases overall mortality, (2) female mortality was increased after HTMV (though this alone does not incriminate DTP), (3) DTP after MV increases mortality in girls (I don't think it does), and (4) MV after DTP reduces mortality in girls PROVIDING THEY HAVE NOT RECEIVED VITAMIN A (but this does not prove that DTP increases mortality, it merely confirms that MV reduces mortality - in both girls and boys, reference 3). The authors' fifth point is merely a rephrasing of their fourth point. I would add, (5) DTP increases mortality in girls and boys after BCG (which is crucially important because this is recommended in the EPI schedule). It is important to emphasise that the non-specific effects occur until the next vaccine is given. It would be better to demonstrate these points clearly and directly (especially points 4 and 5, because they have very important implications for the EPI schedule), without resorting to F/M ratios - which are not convincing.

PA: As mentioned above we have added information for each observation on F/M MRRs on whether the change is likely to be due to increase in female mortality or reduction in male mortality. The reasons for using F/M MRR have been mentioned above. As suggested the part of the discussion on why reduced male mortality does not explain the findings have been rephrased to emphasise the propositions mentioned by professor Shann.

The authors often confuse an increased F/M ratio with increased female mortality. For example, in the Introduction page 4 line 12, reference 5 reports a F/M ratio of about two (which was because of a lower mortality rate in boys who received DTP after HTMV, Table 4) and not a "two-fold increased female mortality" (in reference 5, Tables 2 and 4, female mortality was not higher in the HTMV group between 4 and 10 months). Figure 3 (DTP Observation 4) also states that mortality after HTMV was increased only in girls who had received DTP or IPV after HTMV, and cites reference 5.

PA: We have checked all the statements which could have confused increased F/M ratio with increased female mortality. The wording has been changed in several places.

Non-specific effects of BCG and MV

I suggest that it would be worth devoting a paragraph to the very strong evidence that, until the next vaccine is given, BCG (references 78, 65 and Shann, Arch Dis Child 2010;95:662-7) and MV (references 3 and Shann, Arch Dis Child 2010;95:662-7) reduce mortality from diseases other than tuberculosis and measles. Of course, this paper is about DTP and not BCG or MV, but the strong evidence that BCG and MV have non-specific effects sets the scene for DTP having similar actions.

PA: This was mentioned in the introduction but has now been emphasised more strongly. Furthermore, this documentation has been used to set the scene for the discussion.

Other matters

In the Abstract (page 2 line 7), I suggest "We examined whether whole-cell diphtheria-tetanus-pertussis vaccine (DTP) has ..." In the Data Sources section (page 2, line 10), I suggest "The effect of DTP on mortality up to the next vaccination was assessed in all studies where DTP was given after BCG and there was prospective follow-up after ascertainment of vaccination status." Under Setting (page 2, line 13, I think "high-mortality" is preferable to "low-income"; vaccines have important non-specific effects on mortality only in high-mortality regions (where most deaths are caused by infection), and some countries with a low income have low child mortality (for example, Vietnam and Sri Lanka, as well as Kerala in India).

PA: Modified as suggested and "low-income" has been changed to "high-mortality" throughout the paper.

On page 4 line 32, reference 78 does not make any comment about the ethics of randomised trials of DTP. However, the report of the WHO Task Force on Routine Infant Vaccination and Child Survival that met in London in May 2004 states that "Indeed, the task force considered that some trial designs, such as placebo-controlled or studies involving delay in DPT vaccination, would be unethical." - see www.who.int/entity/vaccine_safety/topics/dtp/taskforce_report.pdf.

PA: The reference has been changed

On page 5 line 54, I agree with the authors that comparisons of sequential vaccinations should be interpreted cautiously, but I do not agree that this is sufficient reason to emphasise the comparison of female and male mortality rates (in EVERY case, the authors have to demonstrate that a high F/M ratio is not due to reduced mortality in boys).

PA: As mentioned above we have attempted to provide this evidence in EVERY case. Furthermore, we have tried more strongly to emphasise that the comparison of sequential vaccinations (because mortality is increased for DTP after BCG rather than reduced as expected) and the comparison of female-male mortality ratios in conjunction suggest that mortality is increased for DTP-vaccinated girls.

Supplementary Table 2 is confusing because (1) it presents only mortality ratios, and (2) it is not clear what groups are being compared - for example, are the DTP ratios DTP1 to unvaccinated, DTP1 to BCG (or MV), DTP1 or DTP2 or DTP3 to unvaccinated or BCG (or MV)? The underlying rates should be reported where possible, and the groups being compared should be described.

PA: As suggested the mortality rates or case fatality ratios have been reported and the comparison groups have been described in greater detail in the supplementary table 2. In the footnotes it has been written: "The control group for BCG would usually be unvaccinated children except in the randomised trial of BCG to LBW children (64) where the controls had received OPV according to current recommendations; the control group for DTP would be BCG-vaccinated and a few unvaccinated children except in the study of introduction of DTP (42) in which virtually none of the children had received BCG; the control group for MV would mainly be DTP vaccinated children."

On page 11 line 56, in the discussion about BCG revaccination, we do not know whether there was a reduction in admissions for girls or an increase for boys - because the text (and reference 69) give only ratios (we should be told RATES).

PA: The rates have been added – it may have been both a reduction for girls and an increase for boys.

In the Discussion (page 12, line 25) it is claimed that the two natural experiments suggested an increased mortality in boys, but I am not sure that this statement is justified by the papers cited. In reference 42, the mortality for DTP versus no DTP in boys was 1.56 (0.70-3.48), which is consistent with a 30% REDUCTION in mortality. The second study (reference 43) presents no data by sex - what was the estimate for boys, and does the 95% CI include 1.00?

PA: In the second study the case fatality was 3-fold increased for both DTP-vaccinated boys and girls but not significant for the sexes individually. This has now been mentioned under Observation 2 as unpublished data. We have deleted the reference to possibly increased mortality for boys in the Discussion.

On page 14, line 53, I suggest that delaying DTP until just before MV at 9 months may not be as effective as giving DTP as at present, but giving MV earlier (just after the three primary doses of DTP). This would continue to give early protection against diphtheria, tetanus and pertussis, and may increase the beneficial non-specific effects of MV (if they are enhanced by the presence of maternal antibodies); a booster dose of MV could then be given after the DTP booster in the second year of life to reverse the adverse effect of DTP and increase protection against measles. It would therefore be of very great interest to randomise children to receive either (1) the standard EPI schedule of BCG at birth, DTP at 6, 10 and 14 weeks, MV at 9 months, and DTP booster at 18 months, or (2) BCG at birth, DTP at 6, 10 and 14 weeks, MV at 18 weeks, DTP booster at 18 months, and MV at 19 months. Now that a booster dose of DTP is recommended in the second year of life, two primary doses of DTP might be enough, which would allow the first dose of MV to be given even earlier (when more infants would have maternal antibody). This suggests randomising to (1) the standard EPI schedule, versus (2) BCG at birth, DTP at 6 and 10 weeks, MV at 14 weeks, DTP at 18 months, and MV at 19 months.

PA: The issue of how to best test the non-specific effects of DTP and the optimal immunisation schedule has now been further discussed. Only few studies have examined the effect of timing of DTP vaccination (55,64), but the data available suggest that early DTP has the strongest negative effects on morbidity and mortality and there might therefore have been an interest in delaying DTP as a potential schedule and to test "pure" effect of DTP by comparing the mortality of children randomised to early DTP vaccination with DTP unvaccinated children randomised to delayed DTP vaccination (=BCG-vaccinated children). However, with the increasing evidence that giving MV earlier is beneficial the best option may be to emphasise this option as a potential schedule and also use it to compare DTP versus MV-vaccinated children. As will be apparent from the comments above about the studies in Gambia and Guinea-Bissau, we are more hesitant about the booster dose of DTP. All the studies suggest that mortality is increased in countries which have implemented a booster dose of DTP. Many countries do not give a booster dose of DTP even though this has now been recommended (again) by WHO. Hence, the discussion of best schedule takes both scenarios with and without a booster dose of DTP into consideration. Furthermore, in the alternative schedule a MV should be maintained at 9 months of age – it will increase protection against measles and it is with this schedule that we have evidence of beneficial effects from previous studies.

References 27, 51 and 53 have the wrong titles. I have not checked all the references, but this needs to be done.

PA: All references have been checked

Conclusion

This is an important study of an extremely important topic. It is outrageous that so little attention has been paid to the evidence that DTP is causing serious harm in high-mortality regions. However, the

paper would be greatly improved by abandoning the use of F/M ratios; at the very least, the underlying mortality rates should be given whenever F/M ratios (or mortality ratios) are used. In addition, I suggest it would be a good idea to mention the strong evidence that BCG and MV have very large beneficial non-specific effects, and to give more emphasis to the strongest evidence that DTP has harmful non-specific effects in girls in high-mortality regions.

PA: All these recommendations have been followed with the exception that we have not totally abandoned the F/M ratios. The reasons for not following this recommendation has been stated above.

There is strong evidence that (1) BCG has beneficial non-specific effects, (2) DTP has harmful non-specific effects when it is given after BCG (as specified in the EPI schedule), and (3) MV has beneficial non-specific effects (for both boys and girls) when it is given after DTP. However, there is only weak evidence that DTP is harmful (for girls) when it is given after MV - and yet that is the interaction the authors have emphasised (it is a weak or non-existent interaction, and the EPI schedule does not recommend giving DTP to infants after MV anyway - though a booster dose of DTP in the second year of life would be given after MV). I would revise the paper to emphasise these points, but this is merely a tentative suggestion that the authors may wish to ignore.

PA: We agree with the first three conclusions and this has been more strongly emphasised in the revised version of the paper in introduction and in the discussion. However, for the reasons stated above we do not share professor Shann's view that there is only weak evidence for saying that there is increased female mortality when DTP is given after MV.

Reviewer: Tobias R. Kollmann

(There are no comments.) The author returned no concerns.

Reviewer: Robert T Chen MD
Medical Epidemiologist
Clinical Trials Team
Division of HIV/AIDS Prevention
CDC, Atlanta

I have no competing interests

General comments:

This is an unusual paper in several ways.

1) The authors (Aaby et al) have summarized in detail a decade-long personal and very uphill quest to explain an unexpected scientific finding: that child survival in certain pediatric vaccinees in low income countries (LIC) is unexpectedly lower than the comparison groups. Due to skepticism over just compilation of observational study findings alone, consistent with recommendations of the Working Group on Non-Targeted Effects, they have recently used small randomized trials to test their hypotheses (with generally supportive findings).

2) The larger vaccine community has been generally reluctant to take the Aaby hypothesis seriously, leading many to simply dismiss the findings without examining the presented evidence or offering alternative hypotheses. The reasons for this dismissal may include:

a. The standard belief/simplification that if vaccines and immunizations are good for public health, they must be an absolute (vs. relative) good. Those who cast any doubts on this absolute good (e.g., Aaby et al) must be either ignorant or "anti-vaccine". *

b. The findings are difficult to believe/understand:

i. Study settings among the poorest in the world (e.g., Guinea Bissau, Haiti, Bangladesh):

1. Difficult to organize good studies logistically
2. Many “bad” things occur in these settings leading to poor child survival anyways.
3. Mortality is a crude final outcome with many possible causes, especially in LDC settings.
4. Difficult to find true peer reviewers. Those that exist know painfully how easy studies can go wrong in such settings and therefore “truth” more elusive.
- ii. These “non-targeted” effects differ by gender (worse in females) for unknown reasons.
- iii. Lack of a known biologic mechanism to explain the findings.
- iv. Dr. Aaby is a demographer by training (vs. medicine or public health), and therefore outside of the usual “fraternity”.
- c. Few others have been willing (to fund) or replicate the study findings. Therefore the authors are by and large the only ones working on this question, further aggravating the dismissal/credibility problem. This is despite many new ongoing and planned clinical trials of candidate vaccines vs. poverty-related diseases (PRD) like dengue, malaria, TB in LICs where both routine pediatric vaccination and child survival are tracked.

PA: These comments are very appropriate but we have not entered into a general discussion of why the vaccine community has been unwilling to listen as this would be a very long story. However, we have emphasised in the end of the paper that many of the non-specific effects have now been supported by randomised trials and that it is high time that all effects are tested rigorously by others. (Dr. Aaby is a social anthropologist by training)

Specific Comments:

3) Introduction: Suggest rewording “observational studies have little impact” to “observational studies have historically rank lower in grade of evidence for quality of study design than randomized studies (ref: PMID 10861325). In the Discussion, the authors may want to point out that this assumption has been questioned by some (PMID:19463302), as this ref (and PMID 10861324) suggest, the results of well-designed observational studies are usually highly concordant with that of the RCTs.

PA: Many thanks for these suggestions which have been followed in the revised version of the paper.

4) For all findings with a significant positive or negative association between the vaccine and the outcome of interest, it would be helpful if the analysis examined if there is a non-random clustering of the time interval (e.g., in weeks) between vaccination and the adverse event or not (e.g., febrile seizures cluster ~1 week after live viral vaccines).

PA: This has not been examined in most of the studies. In the study of the introduction of DTP (42) it was noted that the children in DTP group did not die immediately after vaccination the median time to death being 95 days in the DTP group and 80 days in the unvaccinated groups. Something similar has been noted in ref 26. All survival analyses that we have presented have been controlled for proportionality. If there had been non-random clustering mortality rates would not have been proportional. Furthermore, several of the papers on DTP display survival curves where it will be apparent that there was no non-random clustering of death in the DTP group (1,42,53,64). This has now been mentioned under observation I.

5) For the DTP studies, was acellular pertussis vaccine used at all; if so, does the non-targeted effects differ between wP and aP vaccine? The answer may provide some insights into the mechanism of action.

PA: All the studies reported used whole-cell DTP and this has now been emphasised in the abstract. The effect of acellular DTP is not known though it was used in Senegal in the 1990s. The data has not been reported. We have analysed data from Senegal where half had received acellular and half whole-cell (but without knowing the code) – the overall pattern was the same with girls having 50-60% higher mortality than boys after the 2nd and third dose of DTP (59). Hence, I suspect that there is no

big difference between whole-cell and acellular with respect to sex-differential effects. These data are not reported in the paper since all children in Senegal received BCG and DTP1 simultaneously. There is one RCT of acellular pertussis vaccine from Sweden (Storsaeter et al, PIDJ 1988;7:637-45) where four children died and the authors noted this would have been a significantly increased mortality in Sweden if all eligible children in Sweden (who did not receive pertussis vaccine at the time) had been enrolled in the control group.

6) If some of the databases used in the analyses are available for public use, it may allow other researchers (e.g., doctoral students) to confirm the analyses and findings.

PA: The databases have not been placed on data repositories but have been used for teaching in survival analysis on many occasions. It has been mentioned that the databases are available on a request basis for data sharing at our website.

7) The Working Group on Non-Targeted Effects (WGNTe, Ref 104) had recommended at least six possible trials to assess the nonspecific effects of vaccines on mortality. This paper includes the outcomes of two of the six trials. Please mention Ref 104 earlier in the paper (e.g., in Section B. Testing the Hypothesis) so the reader is aware of these six trials and how the two fit within that context. In the Discussion, please also discuss the status of the remaining recommended trials and why we shouldn't await their results before acting.

PA: the suggestions have been followed. The WGNTe has been mentioned in the introduction and the trials mentioned in the text have been referenced with their number in the WGNTe paper. We have conducted trials similar to the first three priorities in the WGNTe paper. Trial 4 test coadministration of MV and DTP in a slightly different ways than we have done in the Agergaard paper (77). Trial 5 is using a DTP without aluminium which is unfeasible without major funding since a new vaccine would have to be developed. Trial 6 is the delayed DTP trial which is disrecommended by WHO and this trial was also discouraged by professor Shann who is the lead author on the WGNTe paper. The status has been mentioned in the discussion.

8) Rather than the authors submitting the current paper, the impact on policymakers may be greater if the recommendations came from the independent WGNTe, especially after the results of more than two of their recommended trials are available.

PA: Since the WGNTe has not conducted this analysis it can not submit the paper and since the data analysis should still be available we are submitting the paper. However, we have passed on the suggestion to the WGNTe that it might be appropriate to comment on the fact that three of the 6 trials have been completed.

9) As noted above, there are many planned trials of candidate vaccines vs. PRD in LIC that will also track child survival as an outcome. The authors may wish to suggest how these researchers can integrate testing of non-target effects hypotheses into such trials.

PA: Many thanks for this suggestion and we have added in the discussion that the many new trials offer possibilities of testing the non-specific effects of both old and new vaccines.

10) Table 1B, suggest rewording to: "Studies not used in the analysis due to simultaneous vaccination (see text)"

PA: changed as suggested.

This is essentially a review of previously published papers, so other than main study design, little details of the original papers are presented.

PA: it has been mentioned in the method section that details of study design will have to be found in the original studies.

This paper presents the results of two of six recommended trials recommended by the Working Group

on Non-Targetted Effects. So while the results to date are supportive, one can not say the research question has been "answered" until the other trials are done.

PA: It has been emphasised that we have completed the three prioritised trials (Trial 1-3). Using a slightly different study design we conducted Trial 3 of DTP booster (at 14 months) and BCG – we used booster DTP at 18 months and as reported in the present paper this had a strongly beneficial effect. Likewise we have completed a trial which resembles Trial 4 (Measles vaccine after DTP) – we tested MV+DTP+OPV versus MV+OPV. As reported in the paper adding DTP had a negative effect on morbidity and growth for girls. The 5th trial recommends testing DTP without aluminium. Since that product is no longer available the study can not be done by a small research group. Trial 6 is delayed administration of DTP. As discussed above that trial is disrecommended by WHO and probably has less potential to modify current vaccination schedules. It has been mention in the discussion how far we have come in terms of completing the agenda of the WGNTTE.

VERSION 2 – REVIEW

REVIEWER	Robert T Chen MD MA Medical Officer Clinical Trials Team Division of HIV/AIDS Prevention Centers for Disease Control and Prevention Atlanta GA, USA No competing interests
REVIEW RETURNED	10/04/2012

REVIEWER	Frank Shann Staff Specialist in Intensive Care Royal Children's Hospital, Melbourne, Australia. Professor of Critical Care Medicine University of Melbourne, Australia. I have no conflict of interest.
REVIEW RETURNED	12/04/2012

GENERAL COMMENTS	<p>The authors have responded clearly to all my comments, and have added important new information - in particular, the new Table 2, and the underlying rates in Supplementary tables 2, 3, 4 and 5. The paper is now much more convincing, with a clear distinction between rates and F/M ratios, and with greater emphasis on the results of the randomised trials.</p> <p>The paper is a superb presentation of a very complicated set of data - but the topic is so important that it warrants this detailed review. The paper demonstrates that, in high mortality regions, it is very important to minimise the time that whole-cell DTP (or penta) vaccine is the most recent immunisation (by giving a dose of a live vaccine such as measles vaccine or BCG 4-6 weeks after the primary course of DTP, and after a booster dose of DTP). I predict that, in the future, this paper will be seen to be a public health classic.</p>
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